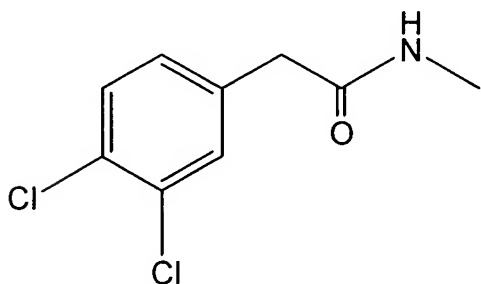


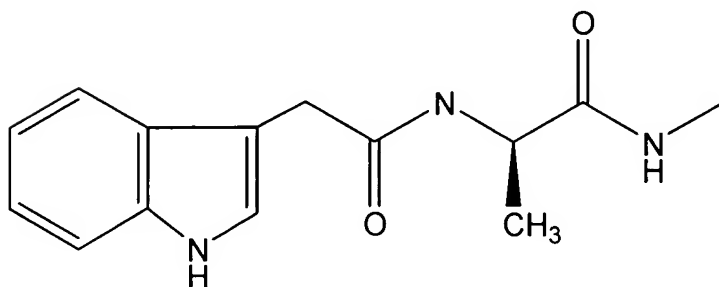
Page 2

Please enter the Sequence Listing attached hereto in both computer readable form on floppy disk and in paper form (7 pages) (**Exhibit 2**) as the Sequence Listing for the subject application.

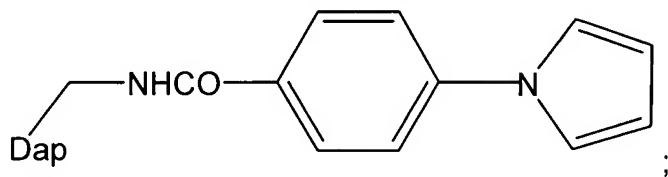
Thus, in some embodiments, the invention is directed to inhibitors of protein kinase C α (PKC α). The inhibitors comprise A-Ala-Arg-Arg-X-B-Hyd-C-D- (SEQ ID NO:1), where A =AcHN-,



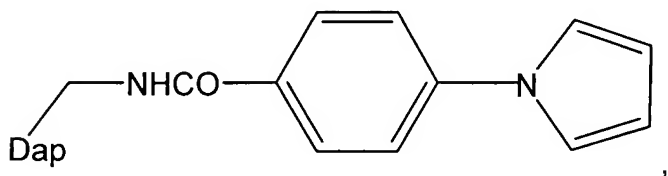
, or



X=any amino acid or amino acid mimetic; B=Ala or a diaminopropionic acid (Dap) derivative having the formula



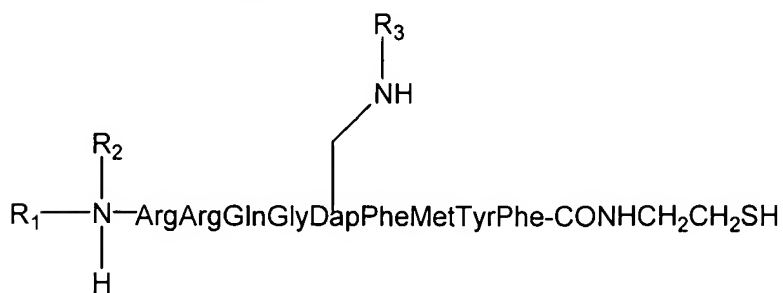
Hyd=Phe, Leu or Ile; C=Arg or Lys; and D=Ala or a Dap derivative having the formula



wherein any of the amino acids can alternatively be an analogous amino acid mimetic.

Please amend the paragraph beginning on page 6, line 8 as follows:

The invention is also directed to inhibitors of a protein kinase C (PKC). The inhibitors comprise



(SEQ ID NO:17),

where R_1 and R_3 are independently H, Ac, a carboxylic acid from FIG. 4, or an aldehyde from FIG. 5, and R_2 is H, a carboxylic acid from FIG. 4, an aldehyde from FIG. 5, or nothing.

Please amend the paragraph beginning on page 7, line 24 as follows:

FIG. 1 shows libraries **I – IV** used to identify inhibitors of protein kinase C α (PKC α). The precursor molecule and the molecules of Libraries I-IV comprise the amino acid sequence set forth in SEQ ID NO:2).

Please amend the paragraph beginning on page 7, line 25 as follows:

FIG. 2 shows various compounds used in PKC α inhibitor studies. Compounds 1-6 comprise the amino acid sequence set forth in SEQ ID NO:2.

Please amend the paragraph beginning on page 7, line 30 as follows:

FIG. 6 shows a general scheme for the introduction of molecular diversity at specific amino acid residues on the consensus sequence. The Dap residue [(L)-2,3-diaminopropionic acid] side chain serves as a handle for the assembly of molecular diversity. The molecules illustrated in Figure 6 all comprise the amino acid sequence set forth in SEQ ID NO:17.

Please amend the paragraph beginning on page 7, line 33 as follows:

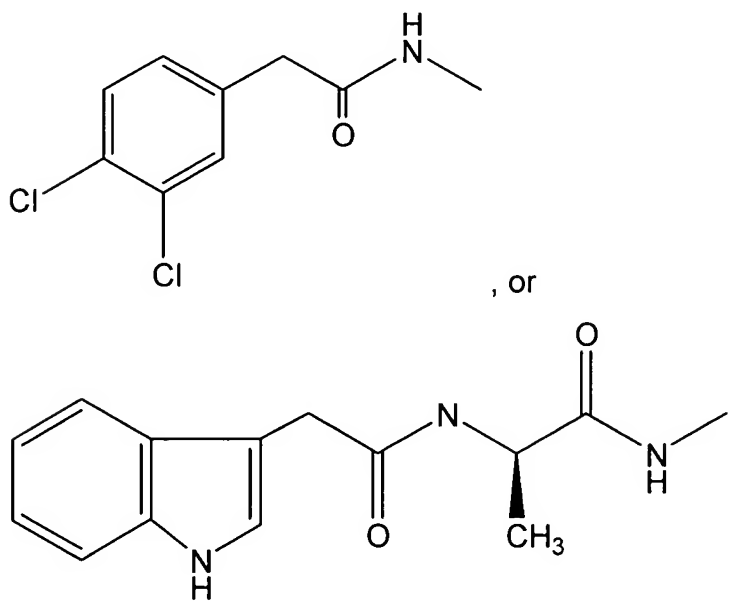
FIG. 7 shows control (compound **A**) and lead peptides (**B - G**) derived from libraries **I - IV**. Compound **H** was previously described (1). Compounds A-G comprise the amino acid sequence set forth in SEQ ID NO:17. Compound H comprises the amino acid sequence set forth in SEQ ID NO:2.

Please amend the paragraph beginning on page 7, line 35 as follows:

FIG. 8 shows a reductive alkylation protocol that furnishes molecular diversity at the N-terminus of peptide **6** while retaining a net positive charge at physiological pH. The latter is an important recognition for the β , δ , and ζ isoforms of PKC within the context of the *p*-nitrobenzoyl-substituted peptide. The molecules illustrated in Figure 8 comprise the amino acid sequence set forth in SEQ ID NO:17.

Please amend the paragraph beginning on page 8, line 9 as follows:

Thus, in some embodiments, the invention is directed to inhibitors of protein kinase C α (PKC α). The inhibitors comprise A-Ala-Arg-Arg-X-B-Hyd-C-D- (SEQ ID NO:1), where A =AcHN-,



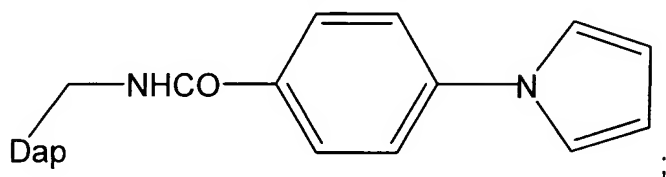
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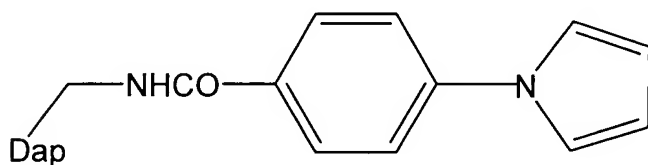
Filed: June 21, 2007

Page 6

X=any amino acid or amino acid mimetic; B=Ala or a diaminopropionic acid (Dap) derivative having the formula

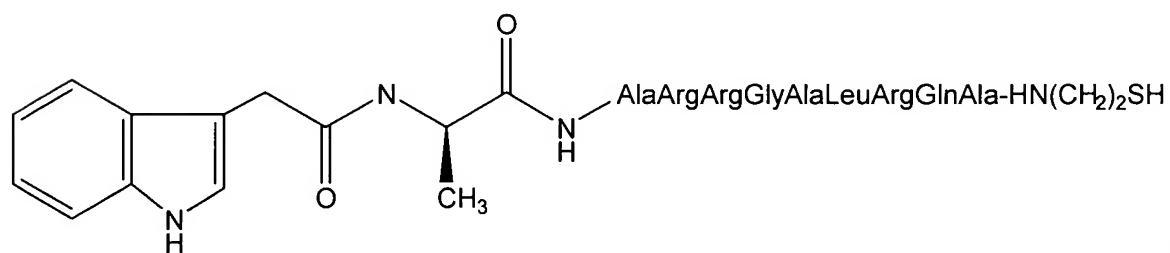


Hyd=Phe, Leu or Ile; C=Arg or Lys; and D=Ala or a Dap derivative having the formula



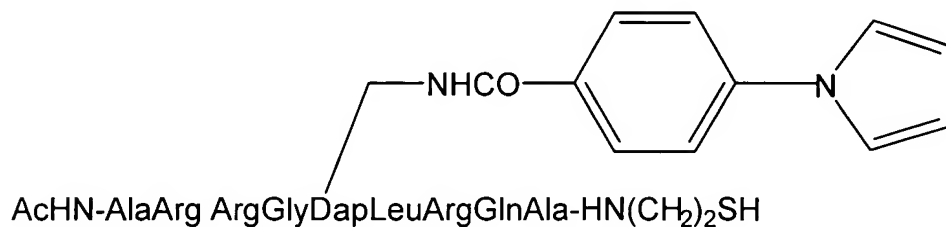
Please amend the paragraph beginning on page 10, line 11 as follows:

In preferred embodiments, the inhibitors comprise, or consist of,



ID NO:2).

(SEQ



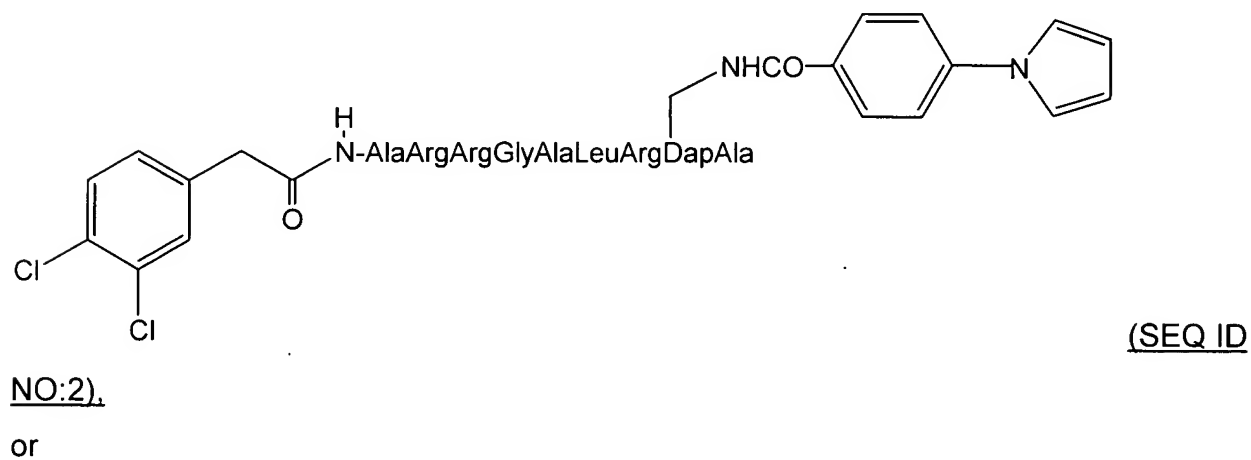
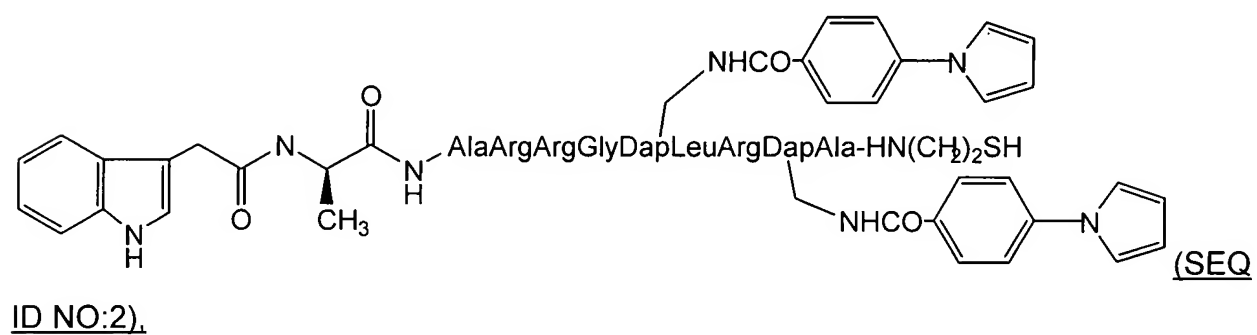
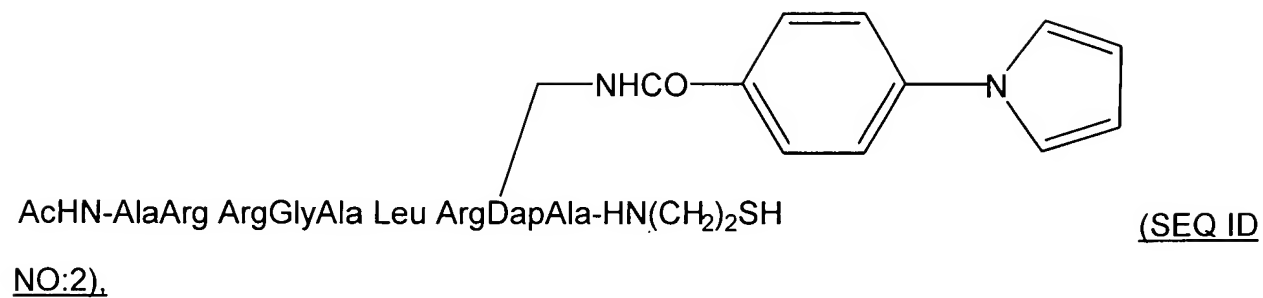
(SEQ ID NO:2).

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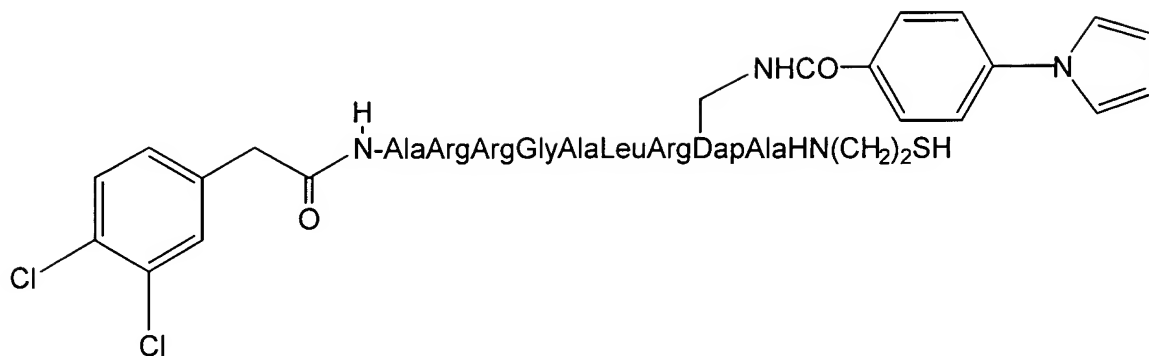


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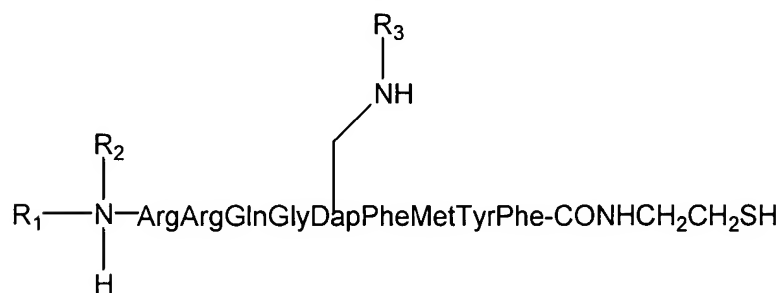


ID NO:2)

(See Example 1).

Please amend the paragraph beginning on page 11, line 6 as follows:

In other embodiments, the invention is directed to inhibitors of a protein kinase C (PKC). The inhibitor comprises



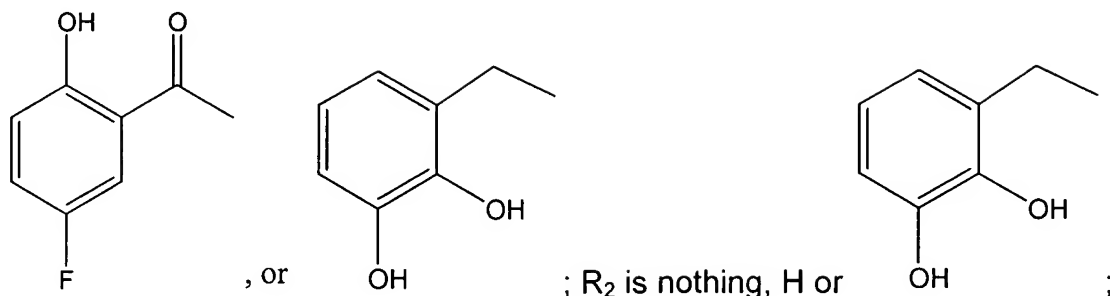
wherein R_1 and R_3 are independently H, Ac, a carboxylic acid from FIG. 4, or an aldehyde from FIG. 5, and R_2 is H, a carboxylic acid from FIG. 4, an aldehyde from FIG. 5, or nothing. In preferred embodiments, R_1 is Ac, H,

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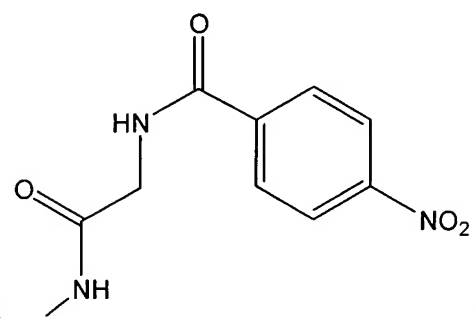
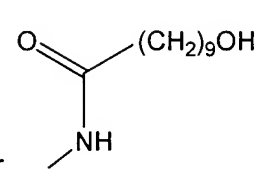
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, or

; R₂ is nothing, H or

;

and R₃ is  or . More preferably, the inhibitor comprises Compound B, Compound C, Compound D, Compound E, Compound F, or Compound G of FIG. 7. Several of these inhibitors are specific for a particular PKC isoform (e.g., Compound F and Compound G of FIG. 7, which are specific for PKC δ and PKC ζ , respectively, or a group of isoforms (e.g., Compound E, which is specific for PKC β I, PKC δ , and PKC ζ). As used herein, an inhibitor is specific for a PKC isoform or group of isoforms if the inhibitor has an IC₅₀ for the PKC <0.1 that of all other of PKC isoforms α , β I, γ , δ , ϵ , θ , η , ι and ζ . Preferably, the inhibitor has an IC₅₀ for the PKC isoform <0.05 that of any other PKC isoform. More preferably, the inhibitor has an IC₅₀ for the PKC isoform <0.01 that of any other PKC isoform.

Please amend the paragraph beginning on page 15, line 23 as follows:

In some of these embodiments, the PKC is PKC α . Where the PKC is PKC α , a preferred consensus sequence comprises LysGlySerHyd(Arg/Lys) (SEQ ID NO:3), where Hyd is Phe, Leu or Ile. In those embodiments, a preferred

consensus sequence having an Ala substituting for the canonical Ser or Thr target residue is AlaArgArgGlyAlaLeuArgGlnAla (SEQ ID NO:2).

Please amend the paragraph beginning on page 15, line 27 as follows:

In other embodiments, the protein kinase is PKC β I and the consensus sequence comprises ArgLysGlySerPheLys (SEQ ID NO:4); the protein kinase is PKC β II and the consensus sequence comprises ArgLysGlySerPheLys (SEQ ID NO:4); the protein kinase is PKC γ and the consensus sequence comprises ArgLysGlySerPheLys (SEQ ID NO:4); the protein kinase is PKC δ and the consensus sequence comprises (Lys/Gln)GlySerPhe(Phe/Met) (SEQ ID NO:5); the protein kinase is PKC ϵ and the consensus sequence is Lys(Met/Lys)Ser(Phe/Ala)(Glu/Tyr/Asp/Phe) (SEQ ID NO:6); the protein kinase is PKC η and the consensus sequence is ArgArgSerPheArgArg (SEQ ID NO:7); the protein kinase is PKC ζ and the consensus sequence is (Arg/Gln/Lys/Glu)(Met/Gly)Ser(Phe/Met)(Phe/Met) (SEQ ID NO:8); or the protein kinase is PKC μ and the consensus sequence is (Gln/Lys/Glu/Met)MetSer(Val/Met/Leu)(Ala/Met/Val) (SEQ ID NO:9).

Please amend the paragraph beginning on page 17, line 7 as follows:

In preferred embodiments of these methods, the protein kinase is a protein kinase C (PKC). Where the protein kinase is PKC α , the preferred inhibitor comprises

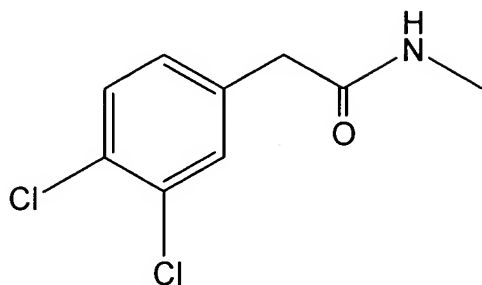
A-Ala-Arg-Arg-X-B-Hyd-C-D- (SEQ ID NO:1), where A- =A α HN-,

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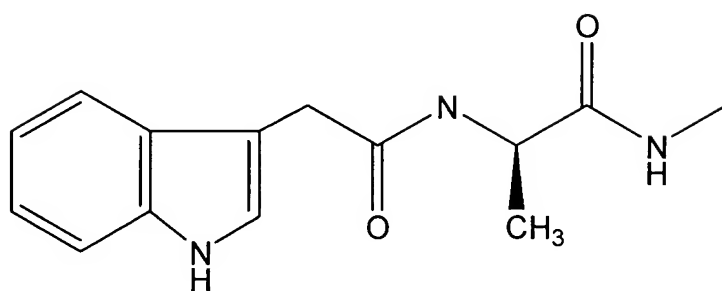
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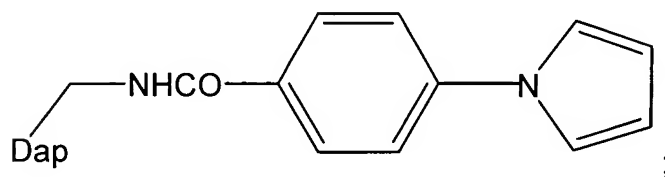


, or



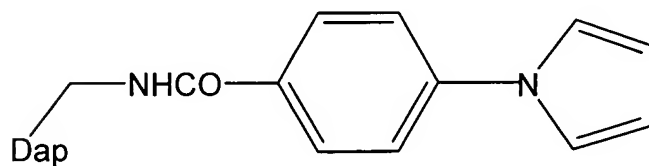
;

X=any amino acid or amino acid mimetic; B=Ala or a diaminopropionic acid (Dap) derivative having the formula



;

Hyd=Phe, Leu or Ile; C=Arg or Lys; and D=Ala or a Dap derivative having the formula



,

wherein any of the amino acids can alternatively be an analogous amino acid mimetic.

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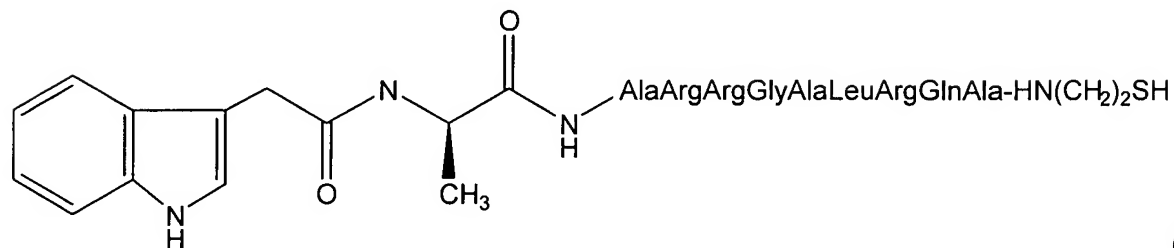
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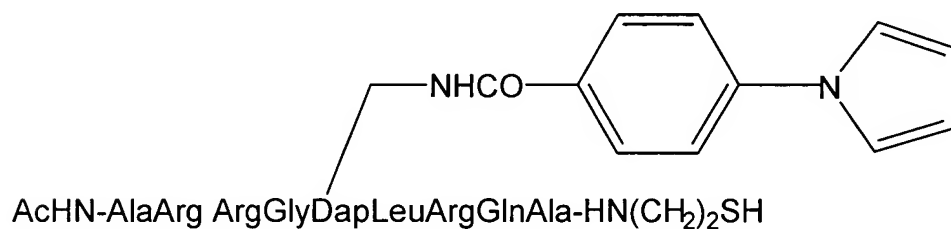
Please amend the paragraph beginning on page 17, line 20 as follows:

Preferred examples of such inhibitors are

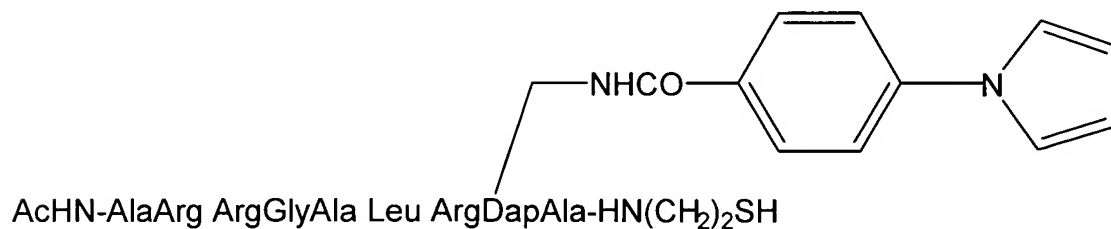


(SEQ

ID NO:2),

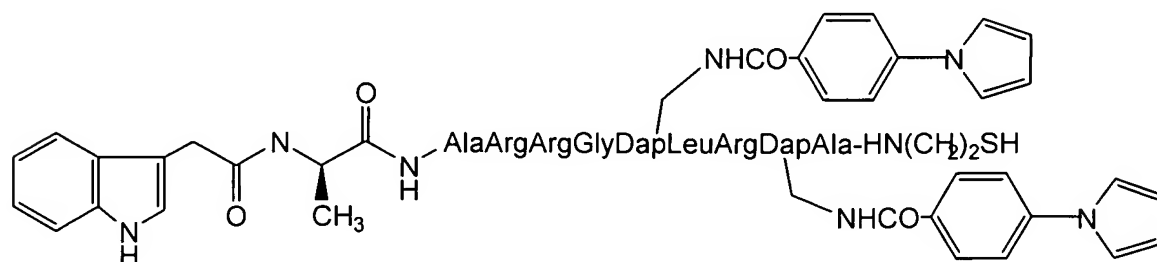


(SEQ ID NO:2),



(SEQ ID

NO:2),



(SEQ

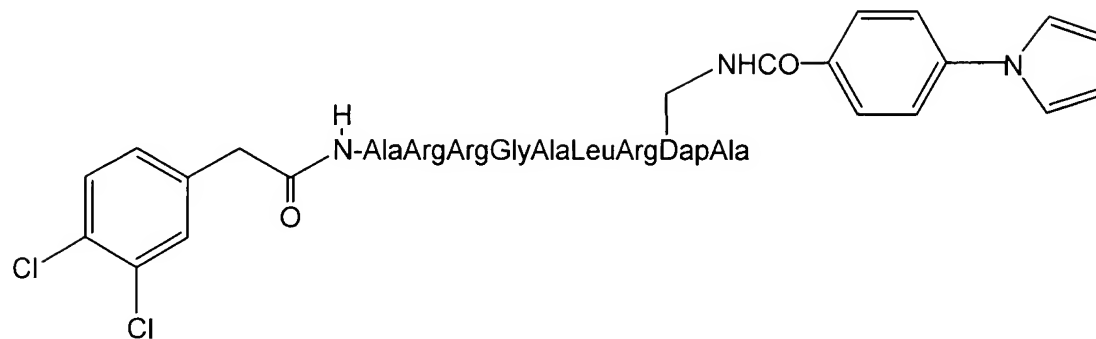
ID NO:2),

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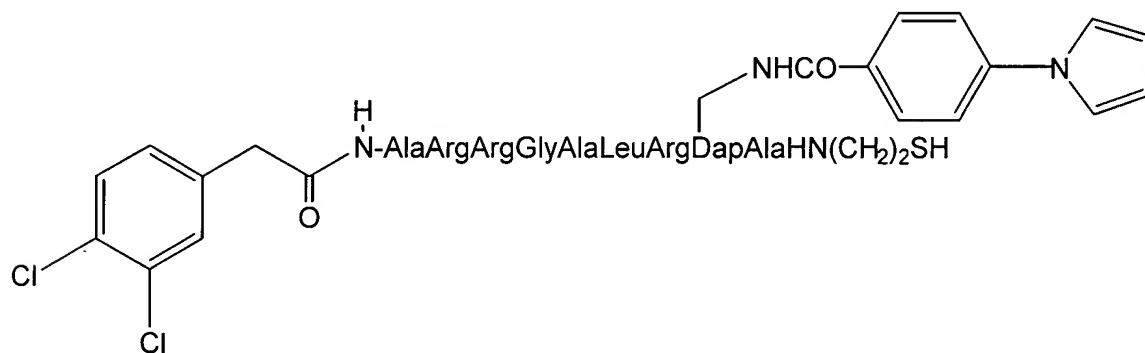
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NO:2).

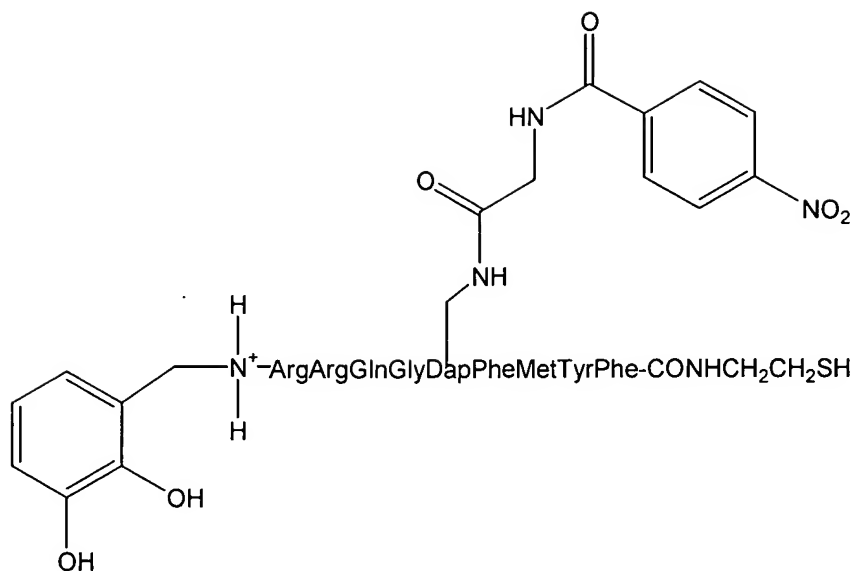
and



ID NO:2).

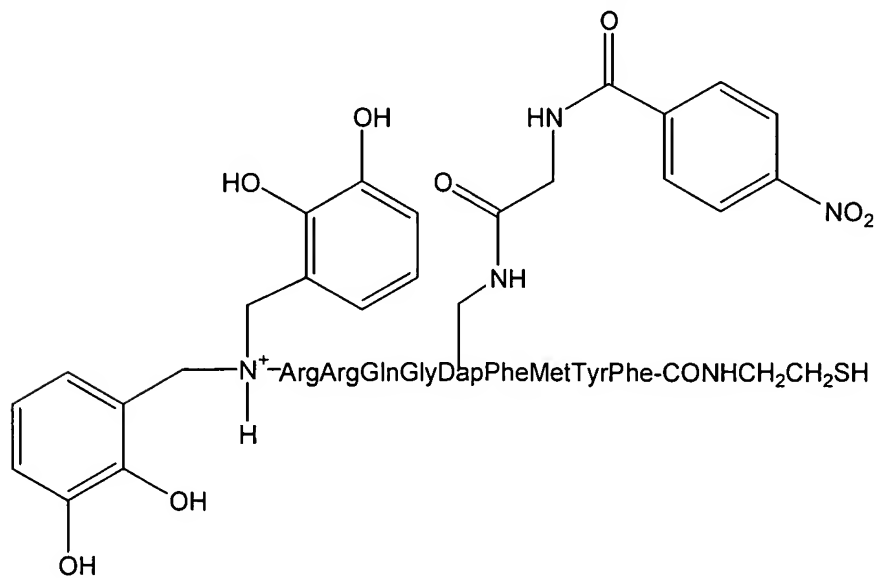
Please amend the paragraph beginning on page 19, line 3 as follows:

Where the protein kinase is PKC δ , a preferred inhibitor is



Please amend the paragraph beginning on page 19, line 5 as follows:

Additionally, where the protein kinase is PKC ζ , a preferred inhibitor is



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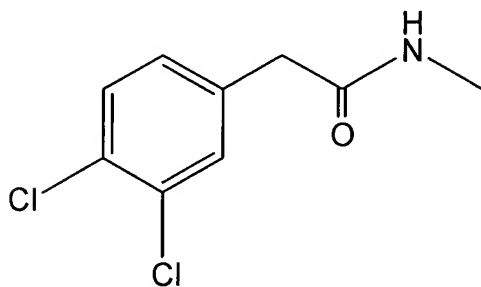
Page 15

Please amend the paragraph beginning on page 20, line 21 as follows:

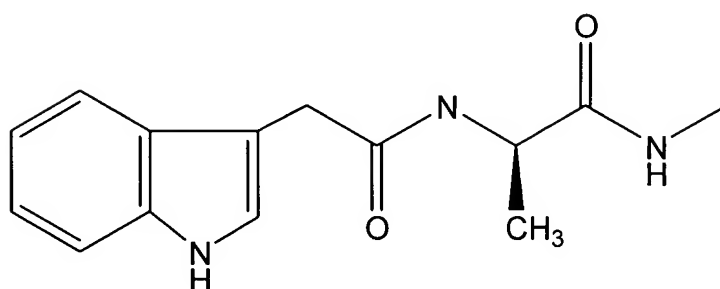
In preferred embodiments, the protein kinase is a protein kinase C (PKC).

Where the protein kinase is PKC α , the inhibitor preferably comprises

A-Ala-Arg-Arg-X-B-Hyd-C-D- (SEQ ID NO:1), where A- =AcHN-,

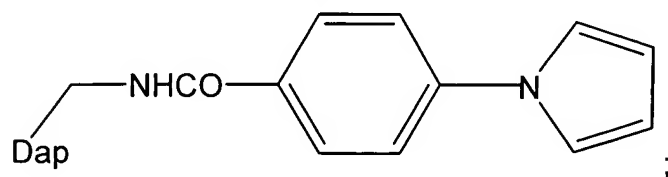


, or



;

X=any amino acid or amino acid mimetic; B=Ala or a diaminopropionic acid (Dap) derivative having the formula



;

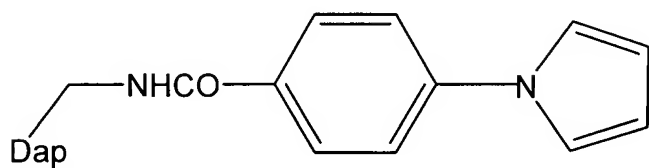
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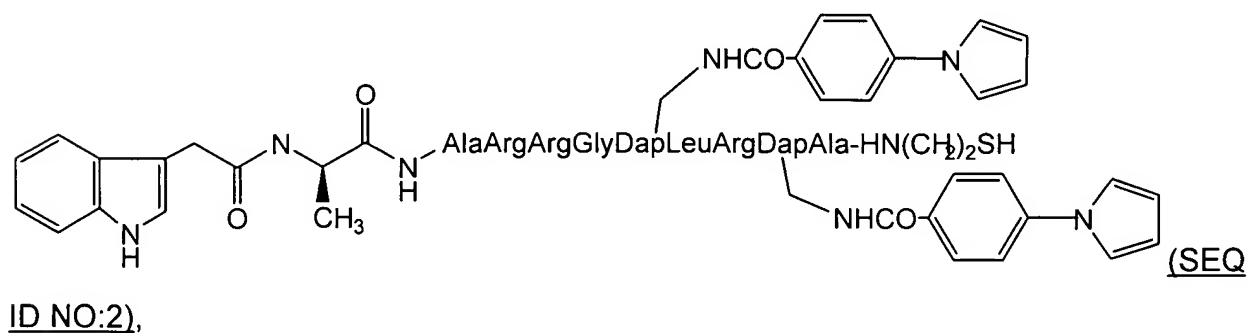
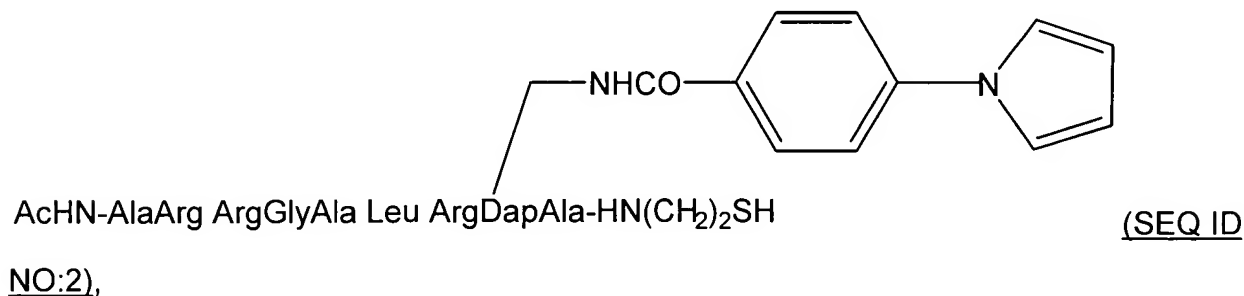
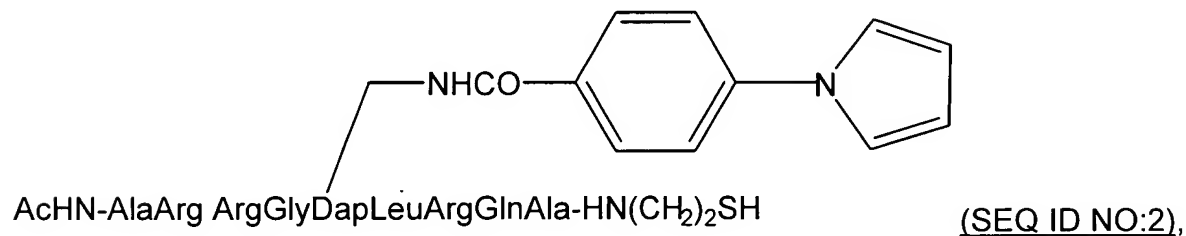
Hyd=Phe, Leu or Ile; C=Arg or Lys; and D=Ala or a Dap derivative having the formula



wherein any of the amino acids can alternatively be an analogous amino acid mimetic.

Please amend the paragraph beginning on page 21, line 8 as follows:

Preferred examples of such inhibitors include,

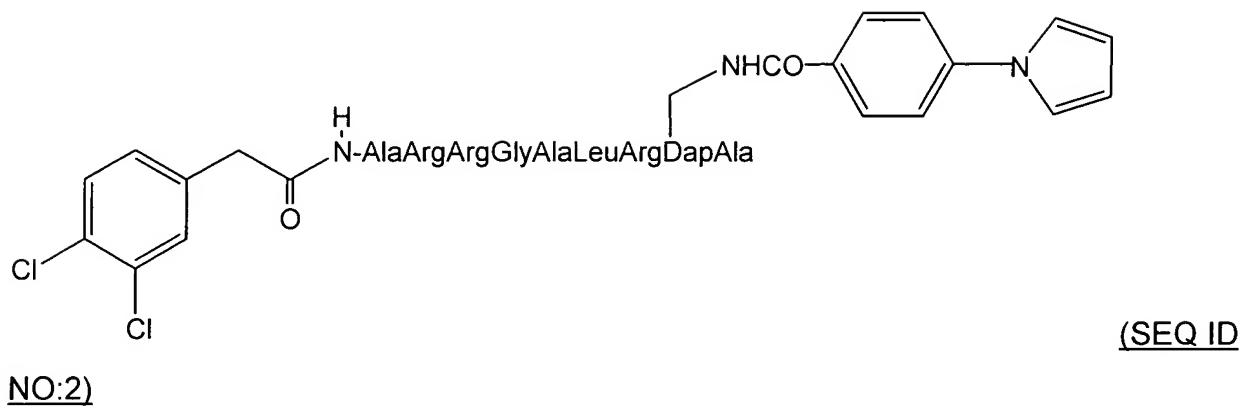


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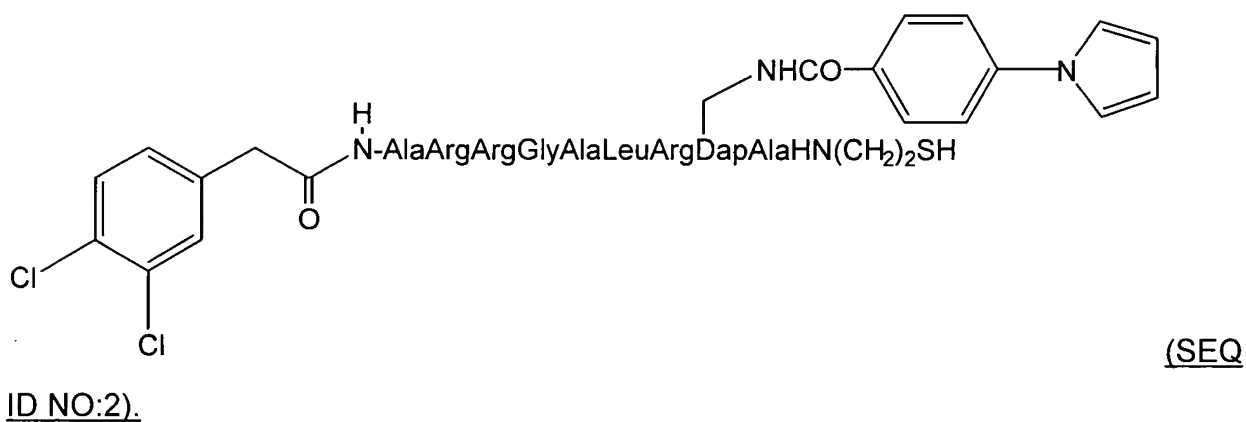
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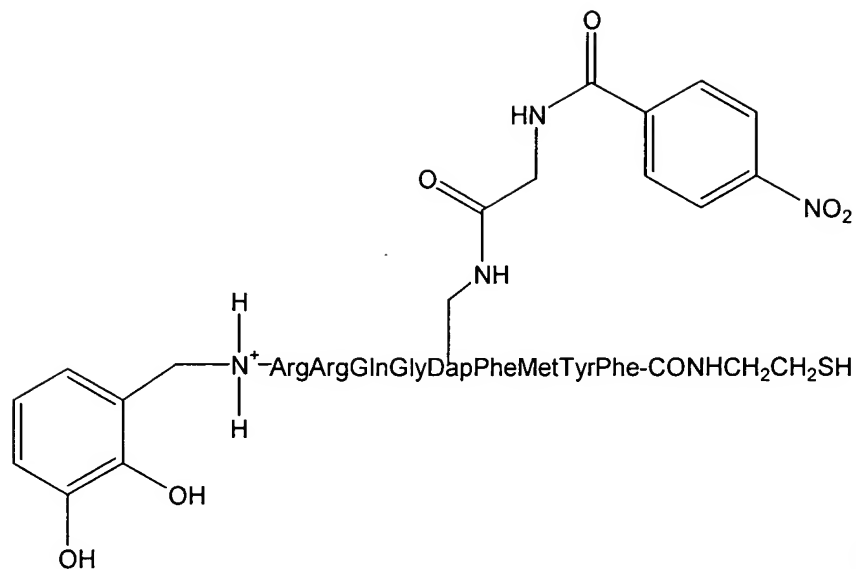


and



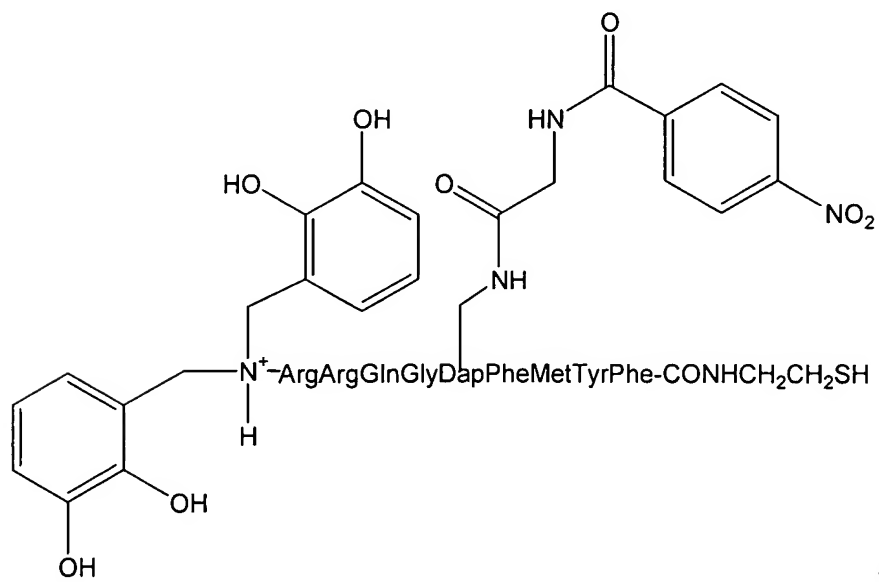
Please amend the paragraph beginning on page 22, line 4 as follows:

Where the protein kinase is a PKC δ , the inhibitor is preferably



Please amend the paragraph beginning on page 22, line 7 as follows:

Where the protein kinase is PKC ζ , the inhibitor is preferably



Please amend the paragraph beginning on page 23, line 24 as follows. Please note that the underlining of "Ala" in this paragraph appears in the original application and does not indicate a change made herein.

We describe herein a library-based strategy that transforms consensus sequences into high affinity ligands in the absence of any tertiary structural information of the protein target. We chose PKC α for our initial studies, an enzyme that is a recognized chemotherapeutic target for several malignant disorders (Nakashima, 2002). The structure of PKC α is not known. A variety of peptide-based inhibitors have been described, the very best of which display IC_{50} or K_i values in the high nM to low μ M range, usually using PKC mixtures (Borowski et al., 2000; Ward et al., 1995; Eichholtz et al., 1993; O'Brian and Ward, 1989; Ricouart et al., 1989; Charp et al., 1988; House and Kemp, 1987). The consensus substrate sequence for PKC α is -Arg-Arg-Lys-Gly-Ser-Hyd-Arg- (where Hyd = Phe/Leu/Ile/) (Nishikawa et al., 1997) (SEQ ID NO:10). We designed the closely analogous nonphosphorylatable peptide Ala-Arg-Arg-Gly-Ala-Leu-Arg-Gln-Ala (SEQ ID NO:2), in which the Ser residue is replaced by Ala. Previous studies have demonstrated that the Arg residues and the hydrophobic amino acid at P-1 promote PKC α recognition (Nishikawa et al., 1997). Consequently, these critical residues were retained and we sought to identify high affinity replacements for presumed nonessential residues or regions on the consensus peptide. In the absence of the 3-dimensional structure of the target protein, three distinct sites on the peptide framework were chosen for the introduction of molecular diversity (libraries I – III [FIG. 1]). For example, a peptide containing (L)-2,3-diaminopropionic acid (Dap) at the former Ala position was synthesized, distributed in equal amounts to individual wells of eight 96 well plates, and then acylated with one of 720 different carboxylic acids to create

library II. Analogous libraries I and III were constructed as well. Following Dap acylation, the side chain protecting groups were removed with trifluoroacetic acid and the peptide then cleaved from the resin with assay buffer (which contains dithiothreitol). The peptide solutions were filtered into deep well plates, stored, and subsequently evaluated for inhibitory potency using a previously described radioactive assay (See Materials and Methods).

Please amend the paragraph beginning on page 28, line 30 as follows. Please note that the underlining of "Protein Kinase C Assay (general)" in this paragraph appears in the original application and does not indicate a change made herein.

Protein Kinase C Assay (general). The peptides Ac-Ser-Phe-Arg-Arg-Arg-NH₂ (for PKC α , β and γ) (SEQ ID NO:11) and acetyl-Pro-Arg-Lys-Arg-Glu-Gly-Ser-Val-Arg-Arg-Arg-NH₂ (for PKC ϵ and ζ) (SEQ ID NO:12) were used as substrates. The K_m values for these peptides are 15 μ M (PKC α) and 5.9 μ M (PKC ϵ), respectively, whereas the V_{max} values are 0.526 μ mol/min-mg (PKC α) and 1.445 μ mol/min-mg (PKC ϵ), respectively.

Please amend the paragraph beginning on page 30, line 8 as follows. Please note that the underlining of "Protein Kinase C α Assay (K_i determination for peptide 6 versus variable Ac-Ser-Phe-Arg-Arg-Arg-NH₂ substrate)." in this paragraph appears in the original application and does not indicate a change made herein.

Protein Kinase C α Assay (K_i determination for peptide 6 versus variable Ac-Ser-Phe-Arg-Arg-Arg-NH₂ substrate) (SEQ ID NO:11). The assay was conducted as described above for peptide 3 versus variable peptide substrate with the

exception that the enzyme solution contained a ten-fold lower concentration of PKC α (0.05 ng/ μ L). The reaction was initiated as described above. After an 18-min incubation at 30 °C, 100 μ L of 6% phosphoric acid was added to each well to stop the reaction (total volume: 150 μ L). Following an additional 5 min incubation at ambient temperature, 75 μ L from each reaction well was transferred into each well of a Unifilter (P81 cellulose phosphate paper) assay plate and washed four times with 0.1% phosphoric acid in water. Scintillation solution was added to each well and 33 P-incorporation measured by scintillation counting with a MicroBetaTM TriLux & MicroBeta JET (Perkin Elmer). IC_{50} values were calculated using GraFit (Erithacus Software Limited) and K_i values were calculated using Enzyme Kinetics, SigmaPlot (SPSS Inc.)

Please amend the paragraph beginning on page 32, line 4 as follows:

Members of the PKC family of enzymes have been implicated as participants in a wide variety of cellular phenomena. For example, the α , β , and ζ isoforms are thought to serve as key players in motility. We have prepared potent, yet exquisitely selective, active site-directed inhibitors for these PKC isoforms in order to explore their role in the signaling pathways that contribute to cofilin phosphorylation. The inhibitors were derived from a starting consensus sequence peptide (RRQGAFMYF) (SEQ ID NO:13), which displays modest affinity and little selectivity for the individual PKC isoforms. An automated parallel synthesis protocol was applied to the consensus sequence, in which specific sites on the peptide scaffold were modified with unnatural substituents to create libraries of 720 analogues. The libraries were screened for inhibitory activity and subsequently modified at a second site to ultimately create inhibitors with the desired properties. The lead PKC δ inhibitor exhibits a K_i of 8 ± 1 nM

and a selectivity that ranges from 25-fold versus PKC ϵ to greater than 200-fold versus the other PKC isoforms. In an analogous vein, the PKC ζ inhibitor displays a K_i of 3.9 ± 0.2 nM and a selectivity of between 400 to nearly 3,000-fold versus other members of the PKC family. To the best of our knowledge, these compounds are the most PKC isoform-selective inhibitors described to date and represent the first examples of selective inhibitors that target specific members of the atypical and novel classes of PKC.

Please amend the paragraph beginning on page 37, line 30 as follows. Please note that the underlining of "Synthesis of Ac-Pro-Arg-Lys-Arg-Gln-Gly-Ser-Val-Arg-Arg-Arg-Val(CONH₂)" in this paragraph appears in the original application and does not indicate a change made herein.

Synthesis of Ac-Pro-Arg-Lys-Arg-Gln-Gly-Ser-Val-Arg-Arg-Arg-Val(CONH₂) (SEQ ID NO:14). Fmoc-Val-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ser(t-butyl)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, and Fmoc-Lys(Mtt)-OH were used for the synthesis of the peptide substrate for the novel and atypical PKC isoforms. 0.93 g of substrate was obtained from 2 g of Rink resin (0.6 mmol/g) using a standard solid phase peptide synthesis Fmoc protocol in a total yield of 43%. ESI-MS (m/z) calculated for C₆₃H₁₁₉N₃₀O₁₅ (MH⁺) 1536.81, Found 1537.15.

Please amend the paragraph beginning on page 41, line 25 as follows. Please note that the underlining of "Protein Kinase C Assays" in this paragraph appears in the original application and does not indicate a change made herein.

Protein Kinase C Assays. The peptides Ac-Ser-Phe-Arg-Arg-Arg-Arg-NH₂ (for PKC α , β and γ) (SEQ ID NO:15) and Ac-Pro-Arg-Lys-Arg-Glu-Gly-Ser-Val-Arg-

Arg-Arg-Val-NH₂ (for PKC δ , ϵ , θ , η , ι , and ζ) (SEQ ID NO:16) were used as substrates.

Please amend the paragraph beginning on page 42, line 23 as follows. Please note that the underlining of "IC₅₀ determinations of resynthesized inhibitor leads for individual PKC isoforms; Protein kinase C α , β -I and γ ; Protein kinase C δ , ϵ , θ , and η ; and Protein kinase C ι and ζ " in this paragraph appears in the original application and does not indicate a change made herein.

IC₅₀ determinations of resynthesized inhibitor leads for individual PKC isoforms.

Assays were performed in triplicate at pH 7.5 and thermostatically maintained at 30 °C using a Boekel constant temperature device, Protein kinase C α , β -I and γ : 20 μ L assay buffer solution, containing 62.5 mM Hepes (pH 7.5), 50 μ M Ac-Ser-Phe-Arg-Arg-Arg-Arg-NH₂, CaCl₂.2H₂O (2.0 mM) (SEQ ID NO:15), MgCl₂.6H₂O (30.0 mM), EGTA.Na (1.0 mM), PS (50.0 μ g/mL), DAG 10 μ g/mL, cold ATP (300 μ M), supplemented with 55 μ Ci/plate [γ -³³P]ATP for radioactive detection, were added to 20 μ L of a solution containing inhibitor lead at various concentrations (2, 4, 8, 16, 32, 64, 128, 256, 512 nM). 10 μ L enzyme buffer solution containing 20 mM Tris (pH 7.5), PKC isoform (~10 ng/well), 0.5 mM DTT, BSA (375 μ g/ mL), and EDTA.4Na.2H₂O (0.5 mM) was added to initiate the reaction. Reactions and their work-up were carried out as described above. The *IC*₅₀ values for pure compounds as inhibitors were calculated based on the experimental data using GraFit (Erithacus Software Limited). Protein kinase C δ , ϵ , θ , and η : As described for PKC α , β , and γ except that the assay was performed in the absence of CaCl₂. Protein kinase C ι and ζ : As described for PKC α , β , and γ except that the assay was performed in the absence of CaCl₂ and DAG.